Biodegradation of Di-(2-ethylhexyl) Phthalate by Novel Rhodococcus Aetherivorans PFS1 Strain Isolated From Paddy Field Soil

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Research Article

Abstract

Di-(2-ethylhexyl)-phthalate (DEHP) is the phthalate ester frequently utilized as a plasticizer, commonly found in cosmetics, packaging materials, moreover, it has carcinogenic and mutagenic effect on humans. In the current study, we isolated the soil bacterium *Rhodococcus aetherivorans* PFS1 and to assess its DEHP degradation ability in various environmental conditions. The strain PFS1 was isolated from paddy field soil and identified by the 16S rRNA sequencing analyses. The strain PFS1 was examined for its biodegradation ability of DEHP at various pH, temperature, salt concentration, glucose concentration, and high and low concentration of DEHP. Moreover, the biodegradation of DEHP at a contaminated soil environment by strain PFS1 was assessed. Further, the metabolic pathway of DEHP degradation by PFS1 was analyzed by HPLC-MS analysis. The results showed that the strain PFS1 effectively degraded the DEHP at neutral pH and temperature 30 °C, moreover, expressed excellent DEHP degradation at the high salt concentration (up to 50 g/L). The strain PFS1 was efficiently degraded the different tested phthalate esters (PAEs) up to 90%. Significantly removed the DEHP contamination in soil along with native organisms which are present in soil up to 94.66%, nevertheless, the PFS1 alone degraded the DEHP up to 87.665% in sterilized soil. According to HPLC-MS analysis, DEHP was degraded into phthalate (PA) by PFS1 strain via mono (2-ethylehxyl) phthalate (MEHP), then PA was utilized for cell growth. These results suggest that *R. aetherivorans* PFS1 has excellent potential to degrade DEHP at various environmental conditions especially in contaminated paddy field soil.

Introduction

Phthalate esters (PAEs) are water-insoluble organic substances, extensively utilized as a plasticizer in polyvinyl chloride (PVC) to increases their plasticity [Heudorf et al., 2007; McCombie et al., 2017]. Due to their xenoestrogenic effect, PAEs are listed as endocrine-disrupting chemicals (EDCs), moreover, it was reported that the PAEs has teratogenicity, carcinogenicity, mutagenicity effect on humans [Sarath Josh et al., 2014]. PAEs are mainly bound with the specific receptor of the endogenous hormones or hindering their metabolism [Craig et al., 2011]. The release and accumulation of PAEs in the environment cause serious environmental and health problems in humans and also hazardous to the aquatic biota [Meng et al., 2014; Zeng et al., 2008a].

The main source of PAEs contamination in arable agricultural land is anthropogenic activities such as the application of agrochemicals [Wang et al, 2013a; Guo and Kannan, 2012], use of wastewater for irrigation, biosolids management and agricultural films usage [Wang et al., 2013b]. The greenhouse films and soil mulching covers contain PAEs, which lead to PAE contamination in agricultural soils. The accumulation of dibutyl phthalate and diethylhexyl phthalate in the agricultural soil where greenhouse practiced are higher than those in non-greenhouse practiced soil [Wang et al., 2002]. The amendment of pesticides and herbicides is one of the main origins of PAEs contamination in agricultural soil [Johnsen et al., 2001]. The PAEs do not only leach from packages of fertilizers, also used as a solvent material in many fertilizers [Wang et al., 2013b]. Previously it was reported that the 22 widely used fertilizers contain the PAEs ranging from 1.2 to 2795 µg/kg [Mo et al., 2008]. The concentration of PAEs in agricultural soils

is increased according to the amount of fertilizers used [Zorníková et al., 2011]. In paddy soils, the amount of PAEs contamination was increased when it was amended with the chemical fertilizers, as well in *Ipomoea aquatica* shoots which grown along with paddy [Cai et al., 2005]. The uptake of PAEs by the crops was found to be positively correlated with the amount of PAEs contamination in agricultural soil [Yin et al., 2003]. This information is about the PAEs contamination in agricultural soils that needs to be considered when chemical fertilization practices.

DEHP is one of the major PAE extensively used as a plasticizer and reported as a hazardous compound by the European community and the United States Environmental Protection Agency [Dargnat et al., 2009; Gao et al., 2004]. Hydrolysis, photolysis, adsorption and biodegradation are the currently available methods to remove the DEHP contamination from the environment, however, the removal of the DEHP from the soil before it enters into the plant, animal and human tissue is important [Chen et al., 2009; Lertsirisopon et al., 2009; Julinova 2012; Benjamin et al., 2015]. Because of DEHP's low hydrolysis and photolysis ratio, microorganisms were commonly used to remove DEHP pollution. Several reports are evidenced that, different bacterial strains can remove the DEHP contamination from the environment. As well as several bacterial strains with DEHP degrading ability were isolated from various environment, such as *Rhodococcus* sp. [Dargnat et al., 2009], *Gordonia* sp. [Nahurira et al., 2017], *Pseudomonas* sp. [Zeng et al., 2004], *Sphingomonas* sp. [Chang et al., 2004], *Arthrobacter* sp. [Wen et al., 2015], *Achromobacter* sp. [Benjamin et al., 2016], *Microbacterium* sp. [Chen et al., 2007], *Pseudoxanthomonas* sp. [Meng et al., 2015], *Gordonia* sp. [Yan et al., 2014], *Acinetobacter* sp. [Xu et al., 2017], *Bacillus* sp. [Qin et al., 2007] etc. However, most of the isolated DEHP degrader can degrade only not more than eight kinds of PAEs. Thus, the finding of strain that can degrade the PAEs under hard conditions is important to eliminate the DEHP contamination from the complex environments. Hence, the current study designed to investigate the DEHP biodegradation ability of soil bacteria belonging to the genus *Rhodococcus*, isolated from paddy field soil under various optimized conditions and contaminated soil environment, and also investigated the different PAEs utilization ability of isolated strain.

Materials And Methods

Chemicals

Di-(2-ethylhexyl) phthalate (DEHP), and other PAEs such as Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Dibutyl phthalate (DBP), Butyl benzyl phthalate (BBP), Dicyclohexyl phthalate (DCHP), Dipropyl phthalate (DPrP), Mono (2-ethylehxyl) phthalate (MEHP), Dihexyl phthalate (DHP), Di-n-heptyl phthalate (DHPP), Di-n-octyl phthalate (DOP), Phthalate acid (PA), Salicylic acid (SA) and Benzoic acid (BA) used in the present study were purchased from SIGMA-ALDRICH Chemical Reagent Co., Ltd.

Isolation and identification of DEHP-degrading bacterial strain

The soil samples from the paddy field were collected from Peruvarappur village (11°26′50″ N, 79°27′58″ E), Cuddalore district, Tamilnadu, South East India. The bulk soil samples were collected in sterile polyethene cover in triplicate, immediately transferred into the laboratory. In addition, 1 g of soil (wet

weight) was applied to a 100 mL of basal medium containing $MgSO_4.7H_2O$ 0.2 g/L, $CaCl_2$ 0.001 g/L, $FeSO_4.7H_2O$ 0.001 g/L, KH_2PO_4 1.5 g/L, $(NH_4)2SO_4$ 2.0 g/L, $Na_2HPO_4.12H_2O$ 1.5 g/L in double-distilled water [pH 7.0] supplemented with 100 mg/L of DEHP. Then the flask was kept for 7 days at 30 °C in a shaking incubator at 180 g. The DEHP concentration was gradually increased from 100 to 600 mg/L in the basal medium after six rounds of acclimation. For enrichment technique, Luria-Bertani (LB) medium containing NaCl 10.0 g/L, yeast extract 5.0 g/L, peptone 10.0 g/L and agar 15 g/L was used. After inoculation into solid LB medium, the plates were cultured at 30 °C for 48 h and the DEHP tolerance single bacterial colonies were purified by streaking on LB plates. Further, each isolated bacterial strain was examined for its DEHP degradation efficacy, the strains were inoculated in basal medium supplemented with 100 mg/L DEHP then incubated for 48 h at 30 °C. The potential isolated strain which showed maximum growth in the basal medium was named PFS1. The residual DEHP concentration in the basal medium was analyzed by gas chromatography. All experimental sets were performed in triplicate [Ting et al., 2018].

The morphological and biochemical characteristics of the PFS1 strain was studied with the BIOLOG Micro-station (BIOLOG Inc., Hayward, CA, USA) [Pigeot-Rémy et al., 2012]. The genomic DNA of the PFS1 strain was extracted with a DNA isolation kit (OMEGA BioTek, Norcross, GA, USA) according to the guidelines of the kit provider. The universal primers 27F (50-AGAGTTTGATCCTGGCTCAG-30) and 1492R (50-GGTTACCTTGTTACGACTT-30) were used for amplification of the 16S rRNA gene. The strain's nucleotide sequences were deposited in GenBank (Ass. No: MK031168) and matched to query sequence using the basic local alignment search method (BLAST). MEGA 6.0 was used to construct a phylogenetic tree using the neighbour-joining algorithm [Tamura et al., 2013].

Effect of pH, temperature and salinity on DEHP degradation

The single factor optimization experimentations were carried to assess the optimized conditions for effective DEHP biodegradation by PFS1 strain. The different pH ranges (4, 5, 6, 7, 8, and 9), different incubation temperatures (10, 20, 30,40, 50 and 60 °C) and different salinity (1, 3, 5, 7, 10, 15, 20, 25, 40 and 50 g/L NaCl) in basal medium enriched with 100 mg/L of DEHP. All experimental sets were kept in a shaker (180 rpm) for 72 h. All studies were repeated in triplicate, and after 72 hours, the remaining DEHP content was calculated using GC analyses.

Effect of inoculum size on DEHP degradation

This is important to check the appropriate inoculum size for the effective degradation of DEHP. Therefore, the effect of different PFS1 (1×10^5) inoculum size (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10%, v/v) on degradation of DEHP (100 mg/L) was examined under optimized conditions. After 48 h of experiments, the aliquots were analyzed in triplicate by GC.

Effect of maximum and minimum DEHP concentration on DEHP degradation

DEHP concentration in natural environments is very low. Thus, it is necessary to assess the biodegradation ability of strain at low and high concentration of DEHP. To assess the biodegradation efficacy of PFS1 strain at maximum and minimum concentration of DEHP, the test was performed with the various initial DEHP concentrations (min, 0.5, 1, 2, 5, and 10 mg/L; max, 50, 100, 150, 200, 400, 600, 800 and 1000 mg/L). After 72 h, the degradation rate was measured by calculating the concentration of DEHP by GC.

Substrate utilization test

The utilization of different PAEs and some organic compound by the test organism is important to validate the effectiveness of the strain under complex PAEs contaminated environment. The isolated PFS1 was cultured in 20 mL basal medium amended with 100 mg/L of subsequent PAEs as the sole carbon source: DEHP, DMP, DEP, DBP, BBP, DCHP, DPrP, DAP, DHP, DHPP, DOP, PA, SA and BA. All experimental setups were carried out in three replicates and kept for 72 h at 30 °C.

Biodegradationof DEHP in contaminated paddy field soil

The biodegradation potential of the PFS1 strain was characterized in a paddy field soil sample collected from where the strain was isolated. To abolish the inherent effects of DEHP, the concentration of DEHP in the collected soil sample was analyzed and calculated. The physico-chemical characteristics of the collected soil sample as detailed in Table 1. Totally, 4 treatment such as treatment 1: non-sterile soil, inoculated with 1×10^7 cells/mL of PFS1 strain, treatment 2: non-sterile soil, without inoculum, treatment 3: sterile soil, inoculated with 1×10^7 cells/mL of PFS1 strain, and treatment 4: sterile soil, without inoculum, was maintained under artificial light condition (16;8, light and dark). In treatment 1 and treatment 2, the soil samples were maintained without sterilization to assess the DEHP degradation efficiency of strain PFS1 along with native microorganisms, however, in treatment 3 and treatment 4, the soil samples were sterilized by autoclave before inoculation of PFS1 strain to avoid the inherent effect of native microorganisms in DEHP degradation. The cell suspension of strain PFS1 (1×10^7 cells/mL) was prepared and inoculated into the soil by drip irrigation method. Treatment 2 and treatment 4 was served as a control for treatment 1 and treatment 3, respectively. All experiments were maintained at 30 °C and a 60% water-holding capacity for 7 days. After incubation, the concentration of DEHP in soil was measured and calculated according to the method described previously [Ren et al., 2016].

Analysis of DEHP degradation pathway

In all degradation experiments, the concentration of residual PAE intermediates was scrutinized using GC (GC-2010 SHIMADZU, Kyoto, Japan) analyses. In brief, the extraction of cultures was done with an equal volume of n-hexane and obtained organic base was centrifuged for 5 min at 10000 g. The residual DEHP and its intermediates degraded by strain PFS1 were determined by HPLC-MS analyses [Chang et al., 2004; Zhang et al., 2016].

Results

Isolation and Identification of DEHP degrading bacterium

The strain PFS1 was isolated from a soil sample collected from the paddy field, and it is more efficient for DEHP-degrading. It is a rod-shaped gram-positive bacterium and forms smooth, lustrous, and orange color colonies on plates containing solid LB medium (Fig. 1). Form the obtained nucleotide sequences of strain PFS1, the neighbour-joining algorithm phylogenetic tree was constructed (Fig. 2), which showed that the strain PFS1 was classified *Rhodococcus* species and it has 99% similarity with *Rhodococcusaetherivorans* strain 10bc312 16S ribosomal RNA gene, partial sequence (GenBank: NR_025208). From this, the isolated PFS1 strain was identified as *Rhodococcusaetherivorans* based on 16S rRNA gene sequence analysis.

Effect of pH and temperature on degradation of DEHP

The influence of pH on the biodegradation of DEHP by PFS1 strain was analyzed by a single factor optimization study. The maximum DEHP degradation was observed at pH 6 to 9 and DEHP was almost degraded completely after 72 hours of incubation. It revealed that the strain PFS1 degraded the DEHP effectively under the alkaline condition when compared with the acid condition. These degradation patterns suggested that alkaline pH is more appropriate for DEHP degradation than acidic condition. The significance of these results, from pH 6.0 to 9.0 showed good DEHP degradation efficiency, which is effective than the pH 4.0 to 5.0, suggesting that the optimal pH for DEHP biodegradation by PFS1is 6.0 to 8.0 (Fig. 3A). The DEHP degradation efficacy of strain PFS1 was assessed at different temperature (10 to 60 °C). The degradation patterns suggest that the ideal temperature for DEHP degradation was 30 °C and lower temperatures showed less favourable for DEHP degradation (Fig. 3B). Under the optimized pH (7), temperature (30 °C) and DEHP concentration (100 mg/L), the DEHP degradation by strain PFS1 was significantly increased, then absolutely degraded after 72 h of incubation (Fig. 4).

Effect of salinity on DEHP degradation

The salinity is comparatively high in industrial wastewater, which discorded in the environment and possibly it reached to arable agricultural land. Here, the biodegradation ability of PFS1 was evaluated at different salt concentration. The DEHP degradation at various salt concentration revealed that the strain PFS1 could withstand a tested maximum salinity up to 50 g/L. The degradation patterns showed that the maximum DEHP degradation of 88.6±2.4% was noticed at 5 g/L after 72 h of incubation. In higher salinity (10-50 g/L), the DEHP degradation rate was comparatively less than the lower salinity (1-7 g/L) after 12 h of incubation, however, it was increased while extending the incubation period up to 72. The results suggest that the PFS1 strain was potentially applicable for the treatment of wastewater without desalination (Fig 5).

Effect of inoculum size on DEHP Degradation

After 48 h of the experiment, the DEHP biotransformation was significantly increased with increasing inoculum size of strain PFS1. The degradation amount of 57.3% at 1% of inoculum size was observed,

further, the degradation ratio was significantly increased with increasing the inoculum size. Moreover, the maximum DEHP degradation of 91% was observed at 10% of inoculum size. When the inoculant size was above 5%, the DEHP removal was reached about 87% (Fig. 6).

Maximum and minimum DEHP concentration for biodegradation by PFS1

It is necessary to determine the biodegradation potential of strain PFS1 at low and high concentration of DEHP. The strain PFS1 could grow and effectively reduce the DEHP at a very high and low concentration of DEHP in the liquid medium. The strain PFS1 was actively degraded at the concentration of 5 mg to 10 mg of DEHP above 91%, however, at the higher concentrations, the maximum degradation of DEHP above 92% was noticed at 50 mg to 400 mg concentration of DEHP (Fig. 7A & B). These result of DEHP degradation patterns signifies that the strain PFS1 was actively regraded the DEHP at 5 mg/L to 400 mg/L concentration of DEHP.

PAEs utilization by the strain PFS1

Different PAEs and organic compounds are present in the contaminated soil along with DEHP, hence, the analyses of PAEs utilization by the strain PFS1 was important to completely remove the DEHP contamination from the soil environment. The strain PFS1 possess the active degradation ability of different PAEs and organic compounds in the liquid medium. The strain PFS1 effectively degraded all tested PAEs at above 90% except DMP and DHP phthalate esters. While, PFS1 was actively degraded the organic compounds such as PA, SA and BA. These results showed that the PFS1 was almost completely degraded the PAEs, moreover, effectively degraded the major intermediates of PAEs, including PA, BA, SA (Fig. 7C).

DEHP degradation by PFS1 in contaminated paddy field soil

The DEHP degradation efficiency of PFS1 strain in a contaminated paddy field soil under 16;8, the light and dark cycle was evaluated. In treatment 1, which inoculated with PFS1 strain in non-sterilized soil showed maximum amount of (94.66%) DEHP degradation, whereas, 48.56% of degradation was observed in treatment 2 which the non-sterilized soil maintained without inoculation. However, treatment 3 which inoculated with PFS1 strain in sterilized soil showed the DEHP degradation of 87.66%, whereas, the degradation of DEHP was recorded as 12.13% in treatment 4 which maintained the sterilized soil without inoculum of PFS1 strain. These results signify that the strain PFS1 has significantly removed the DEHP contamination in soil along with native organism up to 94.66%, however, the PFS1 alone in sterilized soil degraded the DEHP up to 87.66 (Fig. 8).

DEHP Degradation Pathway

The possible pathway of DEHP degradation by the PFS1 strain was determined based on the HPLC analyses. The peaks at 277, 165, and 121 m/z are corresponding to MEHP, PA, and BA, correspondingly (Fig. 9). From this, it was confirmed that the strain PFS1 might use these molecules as a sole carbon source. In this study, MEHP was formed from DEHP through Hydrolyzation, then PA was formed, further,

PA was decarboxylated to form BA, which was further exploited for cell proliferation through the benzoic acid degradation pathway (Fig. 10).

Table 1. Physico-chemical characteristics of collected soil sample

Source	Salinity (%)	pH	TOC (g/kg)	TN (g/kg)	TP (g/kg)	DEHP concentration (mg/kg)
Paddy field	4.64±0.34	7.1±0.84	16.75±2.43	2.16±0.43	0.43±0.094	0.53±0.045

TOC: Total organic carbon, TN: Total nitrogen, TP: Total phosphorus.

Discussion

Microbial bioremediation of pollutants is a possible way to restore our polluted environment, especially the terrestrial ecosystem. Recently, many bacterial species with effective PAEs degradation ability were reported. *Gordonia* sp. exhibited great degradation potential on the DBP and DOP [Jin et al., 2012; Jin et al., 2013]. Wang et al. [Wang et al., 2015] reported the DEHP degradation ability of *Rhodococcusgloberulus* WJ4, similarly, Lu et al. [Lu et al., 2009] examined the DMP, DEP and DBP degradation potential of *Rhodococcus* sp. L4. The soil bacterium *Bacillus subtilis* was exhibited the great biodegradation of DBP, DEP, DPP, DPrP up to 99% in the soil environment [Quan et al., 2005]. Among the various DEHP degraders, *Rhodococcus* species are the most successful candidates with the excellent ability to degrade the DEHP [Kuyukina and Ivshina 2010]. To thrive in polluted environments, most DEHP degrading bacterial strains may change their cell wall and membrane compositions [De-Carvalho, 2010]. In the present study, we isolated effective DEHP degrading bacterial strain *Rhodococcusaetherivorans* PFS1 from chemical fertilizers and pesticides practiced paddy filed soil. In general, several microbial strains were identified from various environments such as river basins, agricultural soil, garden soil, wetlands, etc. Micro and macro plastic debris are the major pollutants in the soil and aquatic ecosystems, moreover, the accumulation of plastic debris in domestic or industrial effluent makes it difficult to treat due to its high salinity (about 5000–6000 mg/L NaCl) [Wu et al., 2008a & b]. Previous reports revealed that the DEHP is a dominant PAE contaminated in various environments [Mackintosh et al., 2006; Cai et al., 2008; Meng et al., 2014], besides, the DEHP is among the most commonly produced PAE and used as a plasticizer [Vitali et al., 1997].

In our study, the strain PFS1 showed excellent tolerance capacity at higher salinity and showed effective DEHP degradation. Several studies showed that the optimum pH and temperature are a significant aspect of the microbial biotransformation of DEHP [Ren et al., 2018 & 2016]. Strain PFS1 showed the effective DEHP degradation at high salinity condition, which may minimize the desalinization process waste remediation, or when it applicable in wastewater irrigated agricultural soil for DEHP degradation. Effective microbial biotransformation is usually mediated by the microbial enzymes, which is active under neutral or slight acidic/alkaline condition [Jin et al., 2012; Nomura et al., 1992]. Most of the identified DEHP

degrading bacterial isolates such as *Arthrobacter* sp. C21 [Wen et al., 2014], *Sphingomonas* sp. PA-02 [Zeng et al., 2008b], *Gordonia* sp. Dop5 [Sarkar et al., 2013], and *Rhodococcus* WJ4 [Wang et al., 2015], showed the high degradation potential at pH 7.0, however, showed negligible tolerance at high or low pH. However, the strain PFS1 exhibited greater DEHP degradation activity from pH 6.0 to 10.0, which is better than pH 4.0 to 5.0. These results suggest that the strain PFS1 is suitable for DEHP degradation at alkaline pH and high salinity. Strain PFS1 also exhibited effective DEHP degradation ability at 30 °C. The high-temperature tolerance capacity of strain PFS1 was consistent with the *Gordoniaalkanivorans* YC-RL2 [Nahurira et al., 2017] and *Bacillussubtilis* 3C3 [Navacharoen and Vangnai, 2011], which able to degrade DEHP at 50 °C. These obtained results indicate that the strain PFS1 was effective to degrade the DEHP in the contaminated environment under various environmental condition.

The degradation of PAEs is mainly involved by the degradation of individual substrates of PAE, as well as these PAE substrates are appeared as a mixture in the environment. The coexistence of PAE substrates in the environment may limit the degradation capability of individual PAE degraders to each substrate, resulted in an attenuated biodegradation process [Desai et al., 2008]. Hence, the evidence concerning the simultaneous PAEs degradation is necessary for the selection and application of efficient microbial strain to the bioremediation purpose. In this study, the strain PFS1 was effectively degraded almost all the tested PAE substrates above 90% except DMP and DHP phthalate esters. The slightly slowed degradation rate of DPP, DBP, and BBP in the substrate mixture condition suggested that substrate competition was affecting their biodegradation [Navacharoen and Vangnai, 2011].

The PA is the main intermediate in PAEs biotransformation including DEHP [Liang et al., 2008], which is a recalcitrant substrate that is supposed to cause kidney damage and cancer [Tan et al., 2013]. Indeed, not all bacteria can perform the transformation of DEHP to the intermediates like PA or BA. For instance, the strain *Gordonia* sp. Dop5 failed to utilize PA [Sarkar et al., 2013]. The strain PFS1 can completely degrade 12 out of 14 tested PAEs. The long-term exposer to a lower concentration of DEHP can cause serious health problems in humans [Hsu et al., 2016]. A lower concentration of DEHP in the environment is difficult to degrade by bacteria, as the concentration is not appropriate for the bacterial growth, or do not induce the expression of the responsible functional genes [Ren et al., 2018; Ren et al., 2016]. The assessment of the degradation ability of microbes at a high concentration of pollutions is important [Nahurira et al., 2011; Ren et al., 2016]. In the present study, the strain PFS1 showed an excellent growth rate at high and low concentration of DEHP and also exhibited greater degradation at the DEHP concentration ranged from 5 mg/L to 400 mg/L.

This is important that the assess the DEHP biodegradation ability of strains at the contaminated environment. Microbial remediation, in comparison to other approaches, will irreversibly depolymerize the composition of contaminants in the setting [Meehan et al., 2000; He et al., 2018,63]. In our study, the strain PFS1 showed 87.66% of DEHP degradation in contaminated soil after 7 days of incubation. The microbial pathway of DEHP degradation has been determined that the DEHP completely de-esterifies to form PA [Liang et al., 2008]. In our study, we observed the metabolic intermediates of PAEs such as MEHP, BA, and PA during the degradation of DEHP by strain PFS1. Initially, MEHP was formed from DEHP

through Hydrolyzation, then PA was formed, further, PA was decarboxylated to form BA [Liang et al., 2008]. BA was used to produce phenol and catechol through ring hydroxylation or -oxidation, which were then metabolised in the tricarboxylic acid (TCA) cycle. [Zeng et al., 2002]. A similar pathway was proposed earlier reports; the strains of *Gordoniaalkanivorans* YC-RL2 [Nahurira et al., 2017], *Pseudomonasfluorescens* FS1 [Zeng et al., 2004], and *Microbacterium* sp. CQ0110Y [Chen et al., 2007].

Conclusion

The efficient DEHP degrading bacterial strain PFS1 was isolated from paddy field soil and identified as *Rhodococcusaetherivorans* by the 16S rRNA gene sequencing. The strain PFS1 effectively degrade the DEHP at pH 7.0 and temperature 30 ∘C, moreover, exhibited significant DEHP degradation at a slight alkaline condition than acidic condition. Remarkably, PFS1 degrades the vast range of PAEs (12/14), moreover, PFS1 exhibited great DEHP degradation in contaminated soil environment. These results suggest that strain PFS1 displays effective potential for the biotransformation of DEHP in contaminated soil settings to restore the soil fertility and quality.

Declarations

Conflict of interest

The authors declare that no conflict of interest.

Author's contributions

YK performed Isolation, Identification, and biodegradation studies, wrote the manuscript and manuscript editing, SS & YK performed statistical analyses and data validation. GP planned and supervised the study.

References

Benjamin S, Kamimura N, Takahashi K, Masai E (2016) *Achromobacter denitrificans* SP1 efficiently utilizes 16 phthalate diesters and their downstream products through protocatechuate 3,4-cleavage pathway. Ecotox Environ Saf 134:172–178.

Benjamin S, Pradeep S, Sarath Josh M, Kumar S, Masai E (2015) A monograph on the remediation of hazardous phthalates. J Hazard Mater 298:58–72. https://doi.org/10.1016/j.jhazmat.2015.05.004

Cai QY, Mo CH, Wu QT, Katsoyiannis A, Zeng QY (2008) The status of soil contamination by semivolatile organic chemicals (SVOCs) in China: a review. Science of the Total Environment 389:209–224.

Cai QY, Mo CH, Zhu XZ, Wu QT, Wang BG, Jiang CA, Li HQ (2003) Effect of municipal sludge and chemical fertilizers on phthalic acid esters (PAEs) contents in *Ipomoea aquatica* grown on paddy soils. Chin J Appl Ecol 14(11):2001–2005. doi:10.3321/j.issn:1001– 9332.2003.11.044

Chang BV, Yang CM, Cheng CH, Yuan SY (2004) Biodegradation of phthalate esters by two bacteria strains. Chemosphere 55:533–538.

Chen CY, Wu PS, Chung YC (2009) Coupled biological and photo-Fenton pretreatment system for the removal of di-(2-ethylhexyl) phthalate (DEHP) from water. Bioresource Technol 100:4531–4534.

Chen J, Li X, Li J, Cao J, Qiu Z, Zhao Q, Xu C, Shu W (2007) Degradation of environmental endocrine disruptor di-2-ethylhexyl phthalate by a newly discovered bacterium, *Microbacterium* sp. strain CQ0110Y. Appl. Microbiol. Biotechnol 74:676–682.

Craig ZR, Wang W, Flaws JA (2011) Endocrine-disrupting chemicals in ovarian function: effects onsteroidogenesis, metabolism and nuclear receptor signaling. Reproduction 142:633–646.

Dargnat C, Teil M, Chevreuil M, Blanchard M (2009) Phthalate removal throughout wastewater treatment plant. Sci. Total Environ 407:1235–1244.

De-Carvalho CCCR (2010) Adaptation of *Rhodococcus* to Organic Solvents. In Microbiology Monographs; Steinbüchel, A., Ed.; Münster: Berlin/Heidelberg, Germany 16:109–132.

Desai AM, Autenrieth RL, Dimitriou-Christidis P, McDonald TJ (2008) Biodegradation kinetics of select polycyclic aromatic hydrocarbon (PAH) mixtures by *Sphingomonas paucimobilis* EPA505. Biodegradation 19:223e233.

Gao D, Li Z, Wen Z, Ren N (2014) Occurrence and fate of phthalate esters in full-scale domestic wastewater treatment plants and their impact on receiving waters along the Songhua River in China. Chemosphere 95:24–32.

Guo Y, Kannan K (2012) Challenges encountered in the analysis of phthalate esters in foodstuffs and other biological matrices. Anal Bioanal Chem 404(9):2539–2554. doi:10.1007/s00216-012-5999-2

He L et al (2018) Comparative analysis biochar and compost-induced degradation of di-(2-ethylhexyl) phthalate in soils. Sci. Total Environ 625:987–993.

Heudorf U, Mersch-Sundermann V, Angerer J (2007) Phthalates: toxicology and exposure. Int J Hyg Envir Heal 210:623–634.

Hsu P, Kuo Y, Leon Guo Y, Chen J, Tsai S, Chao H, Teng Y, Pan M (2016) The adverse effects of low-dose exposure to Di(2-ethylhexyl) phthalate during adolescence on sperm function in adult rats. Environ. Toxicol 31:706–712.

Jin D, Bai Z, Chang D, Hoefel D, Jin B, Wang P, Wei D, Zhuang G (2012) Biodegradation of di-n-butyl phthalate by an isolated *Gordonia* sp. strain QH-11: Genetic identification and degradation kinetics. J Hazard Mater 221–222:80–85. https://doi.org/10.1016/j.jhazmat.2012.04.010

Johnsen K, Jacobsen CS, Torsvik V, Sørensen J (2001) Pesticide effects on bacterial diversity in agricultural soils—a review. Biol Fert Soils 33(6):443–453. doi:10.1007/s003740100351

Julinova´ M, Slavı´k R (2012) Removal of phthalates from aqueous solution by different adsorbents: A short review. J Environ Manage 94:13–24. https://doi.org/10.1016/j.jenvman.2011.09.006

Kuyukina MS, Ivshina IB (2010) Application of *Rhodococcus* in Bioremediation of Contaminated Environments. In Microbiology Monographs; Steinbüchel, A., Ed.; Münster: Berlin/Heidelberg, Germany 16:251–262.

Lertsirisopon R, Soda S, Sei K, Ike M (2009) Abiotic degradation of four phthalic acid esters in aqueous phase under natural sunlight irradiation. J Environ Sci 21:285–290.

Liang DW, Zhang T, Fang HH, He J (2008) Phthalates biodegradation in the environment. Appl Microbiol Biotechnol 80:183–198.

Lu Y, Tang F, Wang Y, Zhao J, Zeng X, Luo Q, Wang L (2009) Biodegradation of dimethyl phthalate, diethyl phthalate and di-n-butyl phthalate by *Rhodococcus* sp. L4 isolated from activated sludge. J Hazard Mater 168:938–943. https://doi.org/10.1016/j.jhazmat.2009.02.126

Mackintosh CE, Maldonado JA, Ikonomou MG, Gobas FAPC (2006) Sorption of phthalate esters and PCBs in a marine ecosystem. Environmental Science and Technology 40:3481–3488.

McCombie G, Biedermann S, Suter G, Biedermann M (2017) Survey on plasticizers currently found in PVC toys on the Swiss market: Banned phthalates are only a minor concern. J Environ Sci Heal A 52:491–496.

Meehan C, Banat IM, McMullan G, Nigam P, Smyth F, Marchant R (2000) Decolorization of Remazol Black-B using a thermotolerant yeast, *Kluyveromyces marxianus* IMB3. Environ Int 26:75–79.

Meng X, Niu G, Yang W, Cao X (2015) Di(2-ethylhexyl) phthalate biodegradation and denitrification by a *Pseudoxanthomonas* sp. strain. Bioresource Technol 180:356–359.

Meng XZ et al (2014) Flow of sewage sludge-borne phthalate esters (PAEs) from human release to human intake: Implication for risk assessment of sludge applied to soil. Sci Total Environ 476–477:242–249. https://doi.org/10.1016/j.scitotenv.2014.01.007

Mo CH, Cai QY, Li YH, Zeng QY (2008) Occurrence of priority organic pollutants in the fertilizers, China. J Hazard Mater 152(3):1208–1213. doi:10.1016/j.jhazmat.2007.07.105

Nahurira R, Ren L, Song J, Jia Y, Wang J, Fan S, Wang H, Yan Y (2017) Degradation of Di(2-Ethylhexyl) Phthalate by a Novel *Gordonia alkanivorans* Strain YC-RL2. Curr Microbiol 74:309–319.

Navacharoen A, Vangnai AS (2011) Biodegradation of diethyl phthalate by an organic-solvent-tolerant *Bacillus subtilis* strain 3C3 and effect of phthalate ester coexistence. Int Biodeter Biodegr 65:818–826.

Nomura Y, Nakagawa M, Ogawa N, Harashima S, Oshima Y (1992) Genes in PHT Plasmid Encoding the Initial Degradation Pathway of Phthalate in Pseudomonas putida. J. Ferment. Bioeng 6:333–344.

Pigeot-Rémy S et al (2012) Bactericidal efficiency and mode of action: A comparative study of photochemistry and photocatalysis. Water Res 46:3208–3218.

Qin H, Lin X, Yin R, Zhang H, Wang J (2006) Influence of an arbuscular mycorrhizal fungi and two bacterial strains on DEHP degradation and growth of mung bean in soil. Acta Scientiae Circumstantiae. J Environ Sci. 26:1651–1657.

Quan CS, Liu, Q, Tian WJ, Kikuchi J, Fan SD (2005) Biodegradation of an endocrine-disrupting chemical, di-2-ethylhexyl phthalate, by Bacillus subtilis No. 66. Appl. Microbiol. Biotechnol 66:702–710. https://doi.org/10.1007/s00253-004-1683-6

Ren L, Jia Y, Ruth N, Qiao C, Wang J, Zhao B, Yan Y (2016) Biodegradation of phthalic acid esters by a newly isolated *Mycobacterium* sp. YC-RL4 and the bioprocess with environmental samples. Environ Sci Pollut Res 23:16609–16619.

Ren L, Lin Z, Liu H, Hu H (2018) Bacteria-mediated phthalic acid esters degradation and related molecular mechanisms. Appl. Microbiol. Biotechnol 102:1085–1096.

Sarath Josh MK, Pradeep S, Vijayalekshmi Amma KS, Balachandran S, Abdul Jaleel UC, Doble M, Spener F, Benjamin S. (2014) Phthalates efficiently bind to human peroxisome proliferator activated receptor and retinoid X receptor α, β, γ subtypes: an in silico approach. *J Appl Toxicol*. Jul;34(7):754-65. https://doi.org/10.1002/jat.2902

Sarkar J, Chowdhury PP, Dutta TK (2013) Complete degradation of di-n-octyl phthalate by *Gordonia* sp. strain Dop5. Chemosphere 90:2571–2577. https://doi.org/10.1016/j.chemosphere.2012.10.101

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729.

Tan L, Ning S, Zhang X, Shi S (2013) Aerobic decolorization and degradation of azo dyes by growing cells of a newly isolated yeast Candida tropicalis TL-F1. Bioresour Technol 138:307–313.

Ting Y, Lei R, Yang J, Shuanghu F, Junhuan W, Jiayi W, Ruth N, Haisheng W, Yanchun Y (2018) Biodegradation of Di-(2-ethylhexyl) Phthalate by *Rhodococcus ruber* YC-YT1 in Contaminated Water and Soil. Int J Environ Environ Res Public Health 15:964. doi:10.3390/ijerph15050964

Vitali M, Guidotti M, Macilenti G, Cremisini C (1997) Phthalate esters in freshwaters as markers of contamination sources-a site study in Italy. Environment International 23:337–347.

Wang J, Luo YM, Teng Y, Ma WT, Christie P, Li Z (2013b) Soil contamination by phthalate esters in Chinese intensive vegetable production systems with different modes of use of plastic film. Environ Pollut 180:265–273. doi:10.1016/j. envpol.2013.05.036

Wang J, Zhang MY, Chen T, Zhu Y, Teng Y, Luo YM (2015) Christie P. Isolation and Identification of a Di-(2-Ethylhexyl) Phthalate-Degrading Bacterium and Its Role in the Bioremediation of a Contaminated Soil. PEDOSPHERE 25:202–211.

Wang XK, Guo WL, Meng PR, Gan JA (2002) Analysis of phthalate esters in air, soil and plants in plastic film greenhouse. Chin Chem Lett 13(6):557–560

Wang XL, Lin QX, Wang J, Lu XG, Wang GP (2013a) Effect of wetland reclamation and tillage conversion on accumulation and distribution of phthalate esters residues in soils. Ecol Eng 51:10–15. doi:10. 1016/j.ecoleng.2012.12.079

Wen Z, Gao D, Wu W (2014) Biodegradation and kinetic analysis of phthalates by an *Arthrobacter* strain isolated from constructed wetland soil. Appl Microbiol Biotechnol 98:4683–4690.

Wen ZD, Wu WM, Ren NQ, Gao DW (2015) Synergistic effect using vermiculite as media with a bacterial biofilm of *Arthrobacter* sp. for biodegradation of di-(2-ethylhexyl) phthalate. J. Hazard. Mater 304:118–125.

Wu Y, Chung A, Tam NFY, Pi N, Wong MH (2008a) Constructed mangrove wetland as secondary treatment system for municipal wastewater. Ecol Eng 34:137–146.

Wu Y, Tam NFY, Wong MH (2008b) Effects of salinity on treatment of municipal wastewater by constructed mangrove wetland microcosms. Mar. Pollut. Bull 57:727–734.

Xu J, Lu Q, de Toledo RA, Shim H (2017) Degradation of di-2-ethylhexyl phthalate (DEHP) by an indigenous isolate *Acinetobacters*p. SN13. Int Biodeterior Biodegrad 117:205–214.

Yan JL et al (2014) Isolation and characterization of a highly efficient DEHP-degrading bacterium. Microbiol China 34:2882–2888.

Yin R, Lin XG, Wang SG, Zhang HY (2003) Effect of DBP/ DEHP in vegetable planted soil on the quality of capsicum fruit. Chemosphere 144(206):159–178. doi:10.1016/S0045- 6535(02)00222-9

Zeng F et al (2008a) Occurrence of phthalate esters in water and sediment of urban lakes in a subtropical city, Guangzhou, South China. Environ Int 34:372–380. https://doi. org/10.1016/j.envint.2007.09.002.

Zeng F, Cui K, Li X, Fu J, Sheng G (2004) Biodegradation kinetics of phthalate esters by *Pseudomonas fluoresences* FS1. Process Biochem 39:1125–1129.

Zeng F, Cui KY, Fu JM, Sheng GY, Yang HF (2002) Biodegradability of di(2-ethylhexyl) phthalate by Pseudomonas fluorescens FS1. Water Air Soil Pollut 140:297–305.

Zeng P, Moy BY, Song Y, Tay J (2008b) Biodegradation of dimethyl phthalate by *Sphingomonas* sp. isolated from phthalic-acid-degrading aerobic granules. Appl Microbiol Biotechnol 80:899–905.

Zhang Y, Chen H, Liu J, Geng G, Liu D, Geng H, Xiong L (2016) Genome sequencing and biodegradation characteristics of the n-butyl benzyl phthalate degrading bacterium-*Rhodococcus* sp. HS-D2. Int Biodeter Biodegr 128:56-62.

Zorníková G, Jarošová A, Hřivna L (2011) Distribution of phthalic acid esters in agricultural plants and soil. Acta Universitatis Agricultura 59(3):233–237. doi:10.11118/actaun201159030233

Figures
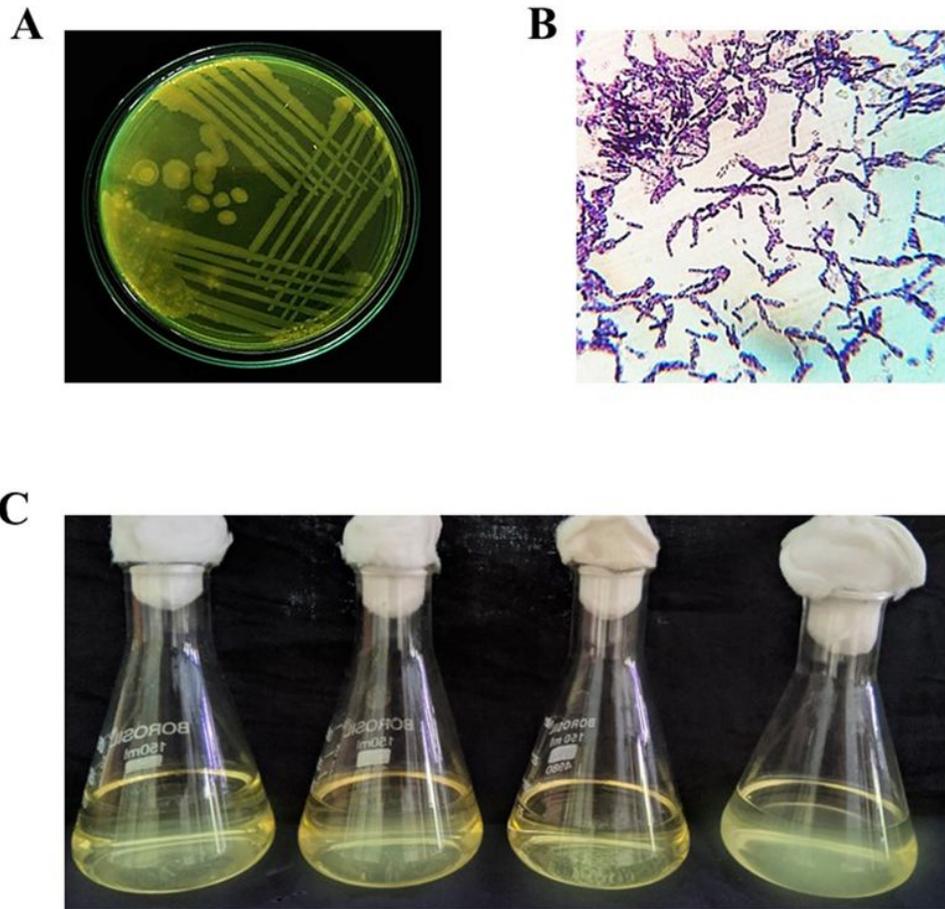
Figure 1

Purification and colony appearance of strain PFS1 on solid Luria-Bertani medium (A). Gram staining and microscopical observation of strain PFS1 (B). Examination of primary DEHP degradation by isolated strains in basal medium supplemented with 100 mg/L DEHP (C).
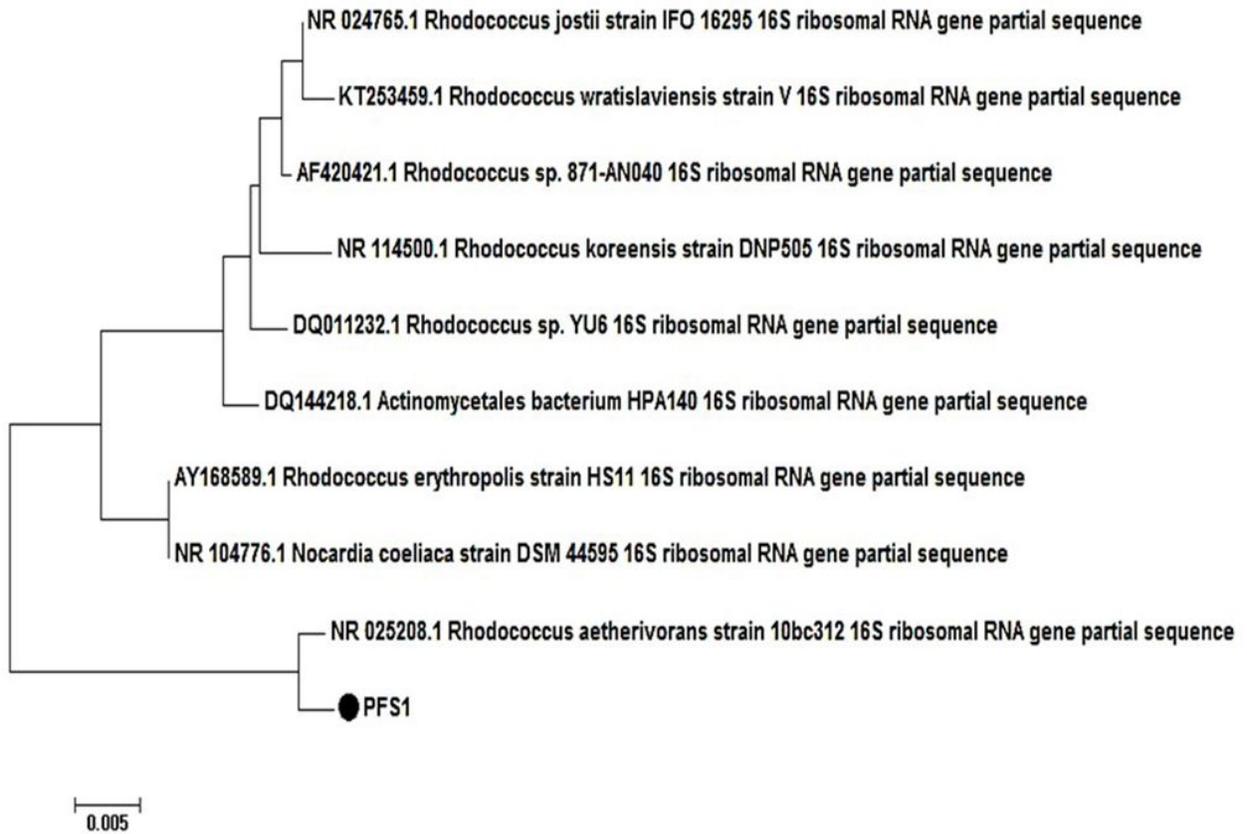
Figure 2

Phylogenetic analysis of strain PFS1 based on 16S rRNA gene sequence analysis. The tree was constructed by the neighbour-joining algorithm method. The scale bar equals 0.005 changes per nucleotide position.
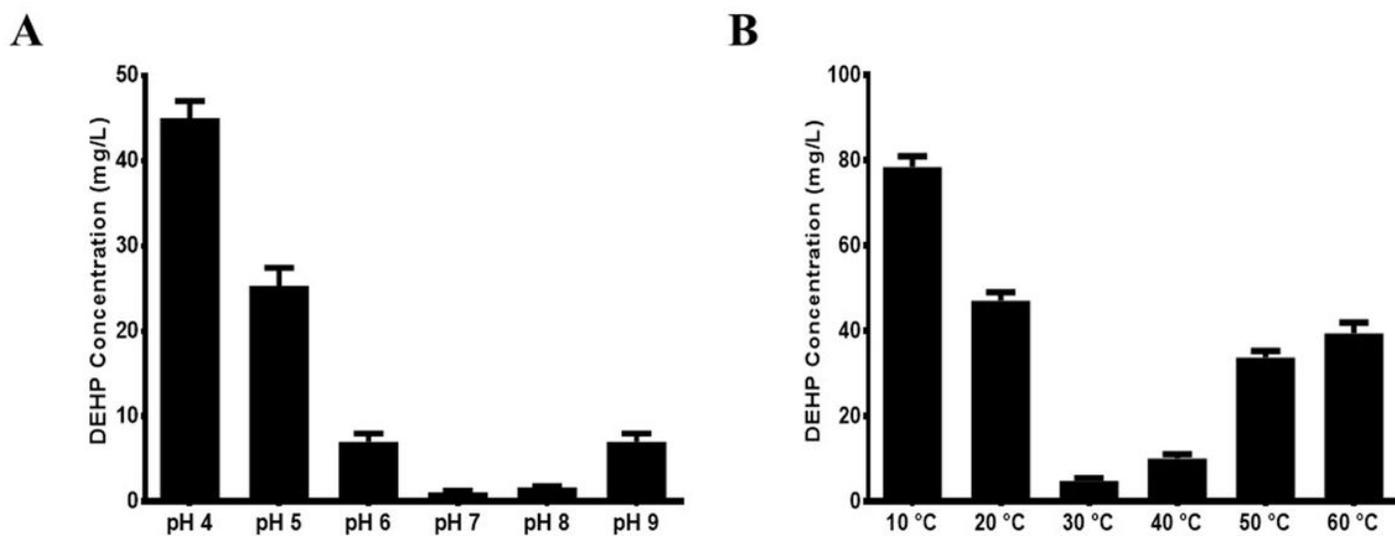
Figure 3

Effect of initial medium pH on DEHP degradation by the isolated strain PFS1 (A). Effect of incubation temperature on DEHP degradation by the isolated strain PFS1 (B). the final concentration of DEHP in the medium was calculated after 72 h of the experiment. Error bars indicate standard deviations of the mean of three replicates.
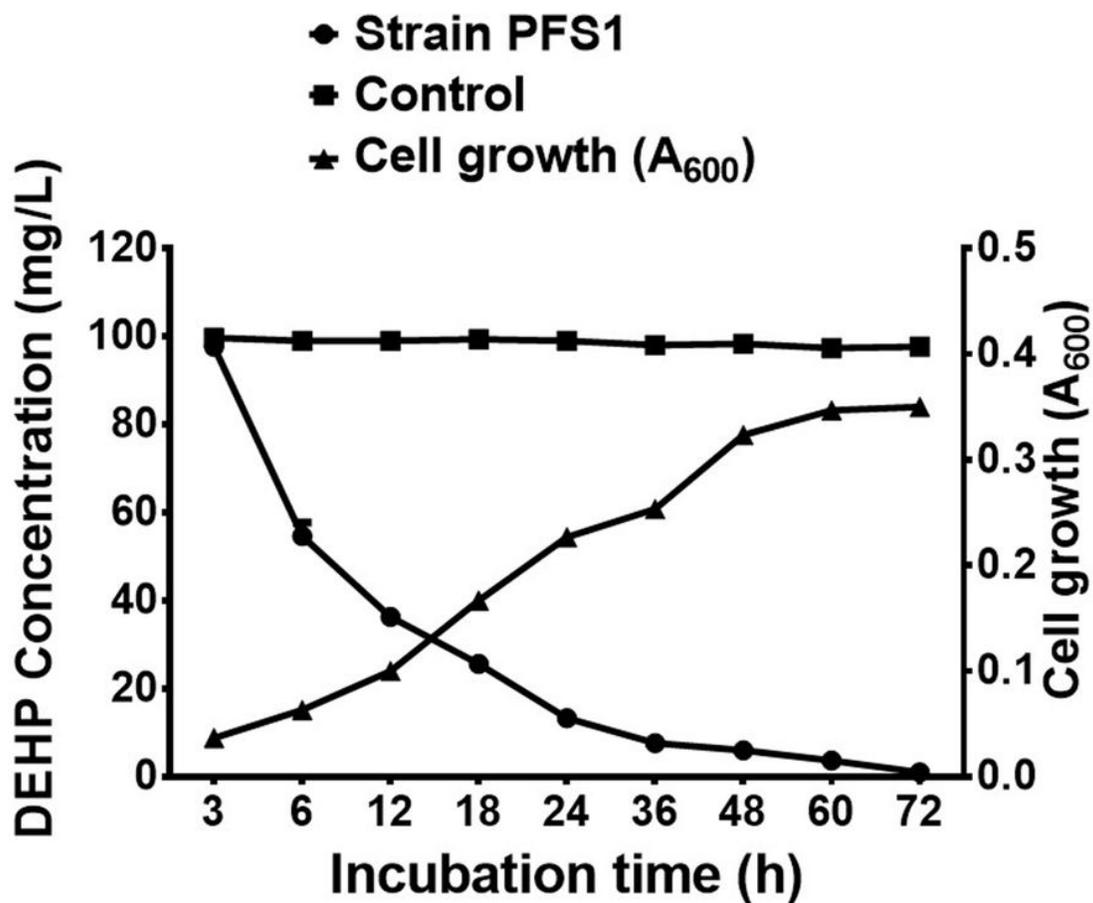
Figure 4

DEHP degradation rate and cell growth of strain PFS1 under optimized conditions of pH and temperature. Error bars indicate standard deviations of the mean of three replicates.
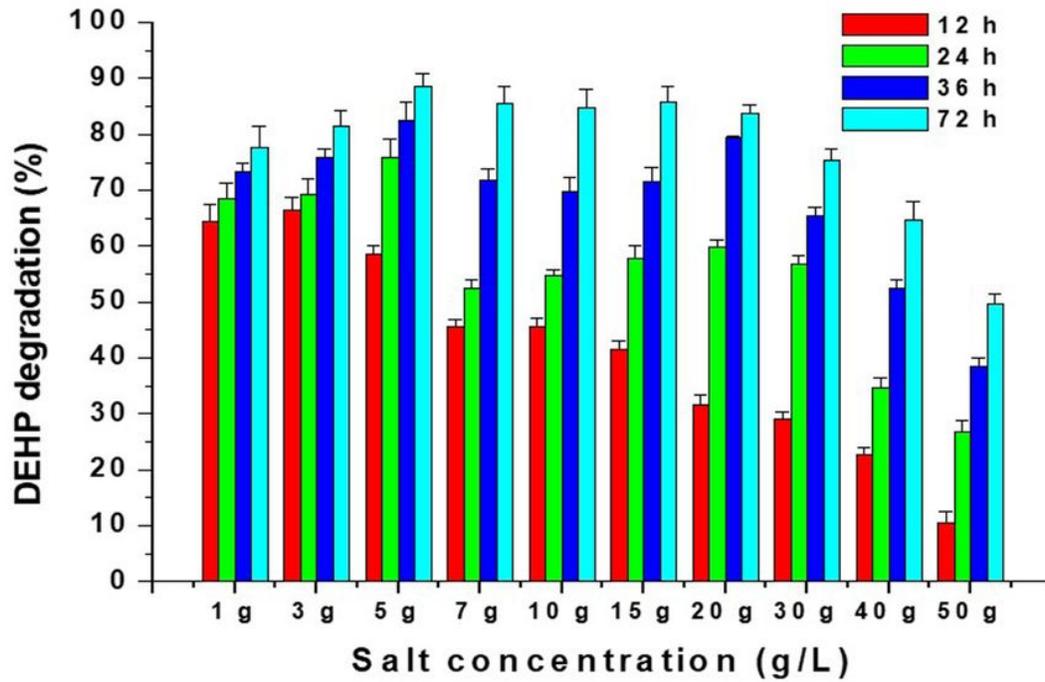
Figure 5

Effect of different NaCl concentration (salinity) on DEHP degradation by strain PFS1. Error bars indicate standard deviations of the mean of three replicates.
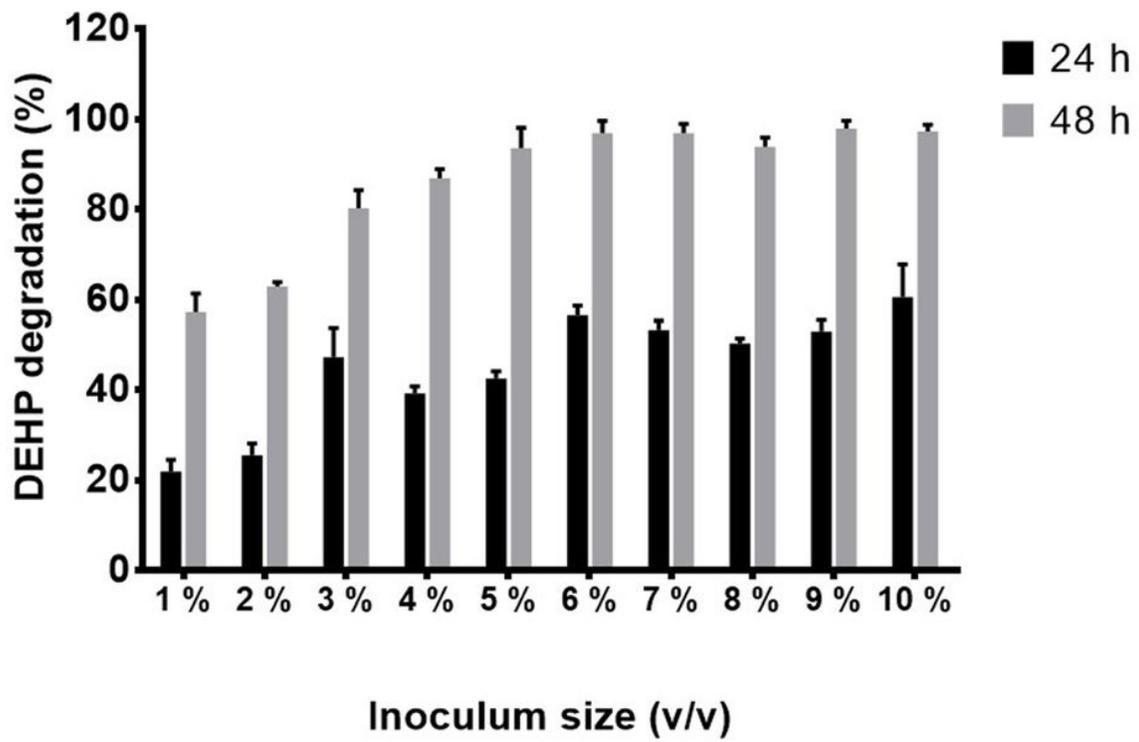
Figure 6

Effect of different initial inoculum size on DEHP degradation by strain PFS1. Error bars indicate standard deviations of the mean of three replicates.
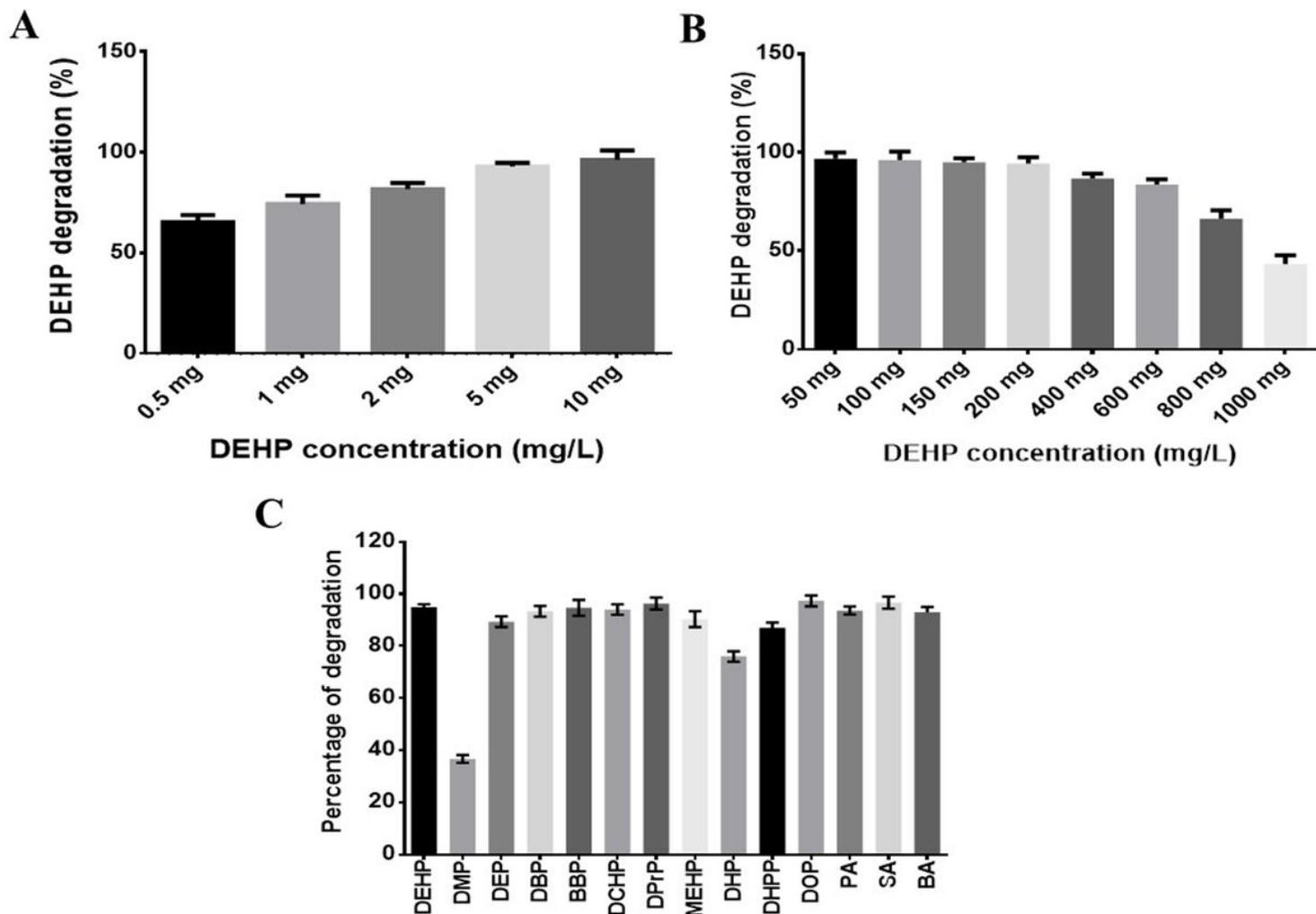
Figure 7

Effect of initial low DEHP concentration (0.5-10 mg/L) on DEHP degradation by strain PFS1 (A). Effect initial high DEHP concentration (50-1000 mg/L) on DEHP degradation by strain PFS1 (B). Individual PAEs degradation efficacy of strain PFS1 (C). Error bars indicate standard deviations of the mean of three replicates.
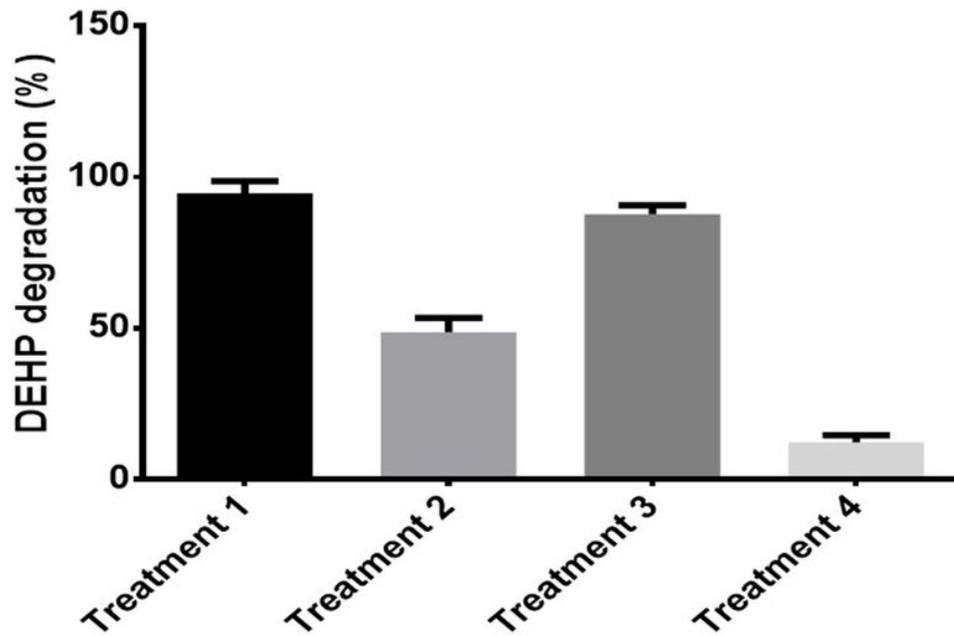
Figure 8

DEHP degradation efficacy of strain PFS1 in contaminated paddy field soil. non-sterile soil, inoculated with 1×10^7 cells/mL of PFS1 strain, treatment 2: non-sterile soil, without inoculum, treatment 3: sterile soil, inoculated with 1×10^7 cells/mL of PFS1 strain, and treatment 4: sterile soil, without inoculum. Error bars indicate standard deviations of the mean of three replicates.
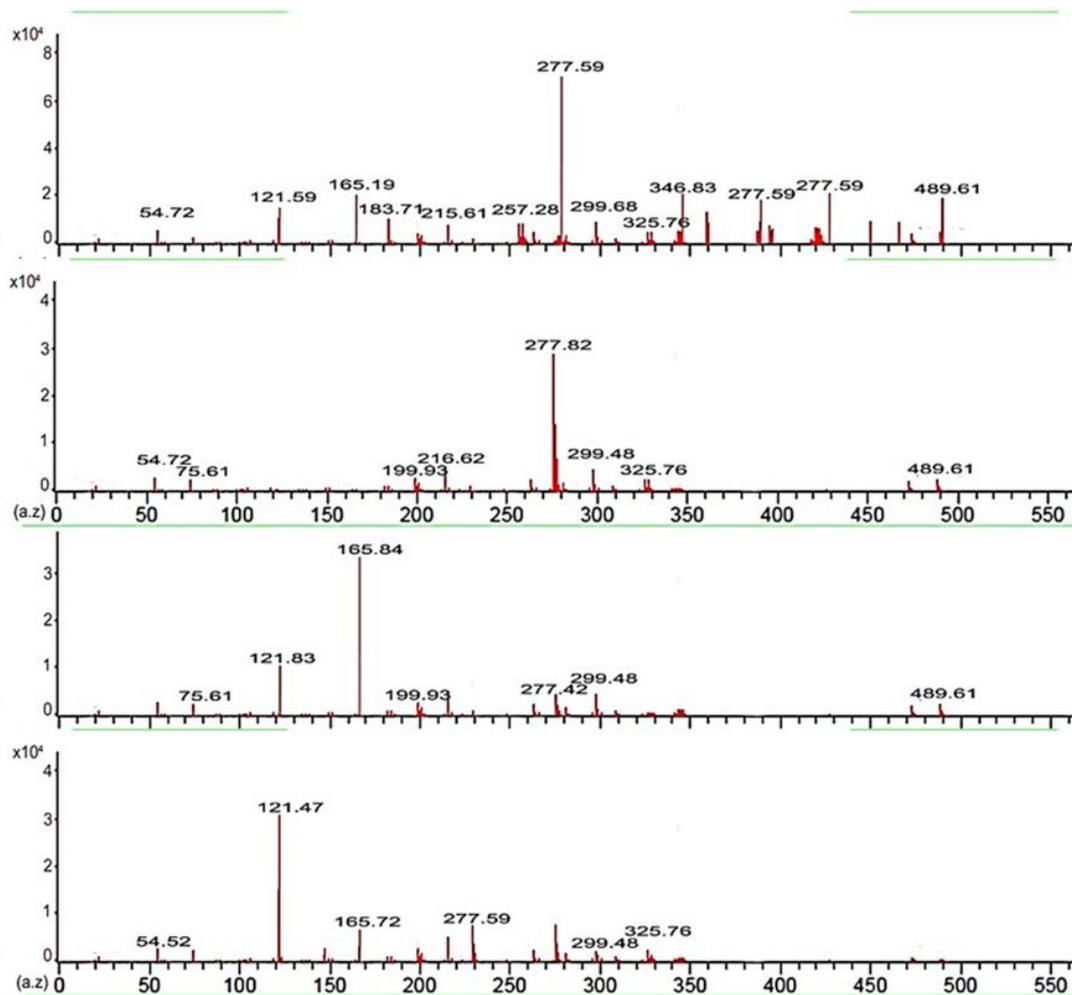
Figure 9

HPLC-MS analysis results of DEHP degradation intermediates. The elevated peaks at 277, 165, and 121 m/z are corresponding to Mono (2-ethylehxyl) phthalate (MEHP), Phthalate acid (PA) and Benzoic acid (BA), respectively.
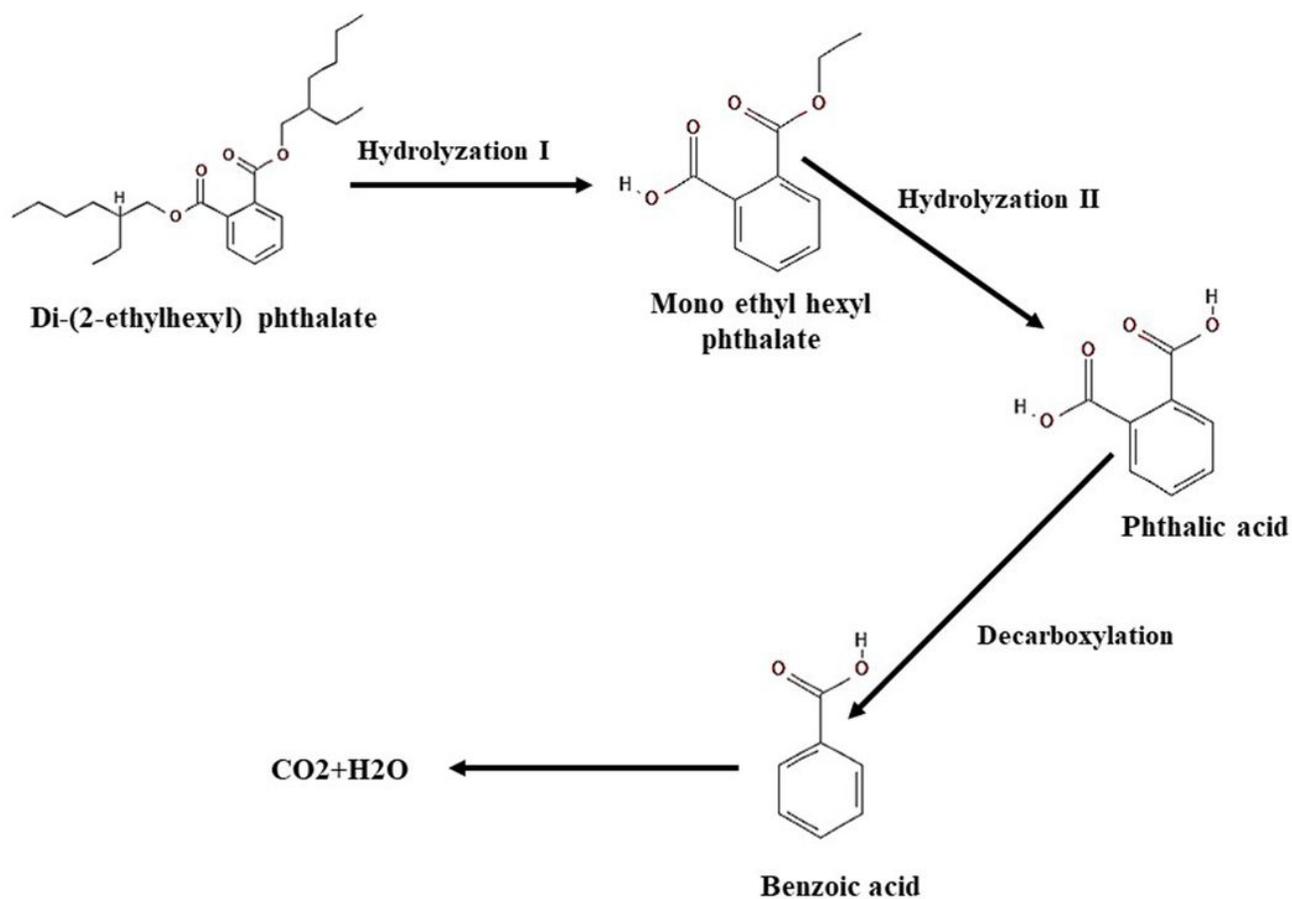
Figure 10

The proposed degradation pathway of DEHP. DEHP was hydrolyzed to phthalic acid (PA) via the intermediate MEHP, further BA was formed from PA via Decarboxylation, then BA was metabolized for cell growth through the benzoate metabolism pathway.